Extraction of Indole Derivatives from Pharmaceutical Drugs, and the Synthesis, Characterization and Biological Evaluation of Their 3d-Transition Metal Complexes

SYNOPSIS

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Synopsis of Research Work for Ph.D. Degree in the Faculty of Science(Chemistry)

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a. Purpose and Need of the Study

Indole scaffolds have long been recognized as "privileged structures" in medicinal chemistry, forming the core of numerous pharmaceutical drugs and natural bioactive compounds. The indole ring system (a fused benzene–pyrrole structure) imparts versatile binding capabilities and bioactivity, as evidenced by drugs like **indomethacin** (a NSAID with an indole acetic acid structure) and essential biomolecules such as **tryptophan** (an indole-containing amino acid). In recent years, indole derivatives have demonstrated efficacy across diverse therapeutic areas – from anticancer agents (e.g. sunitinib) to antimicrobials – even effective against drug-resistant pathogens. This broad spectrum of activity, including anti- inflammatory, antimicrobial, antioxidant, and neuropharmacological effects, underscores the critical role of indole frameworks in addressing major health challenges. However, the **sustainable sourcing** and utilization of these indole- containing compounds remain pressing issues. Traditional chemical synthesis or extraction of active indoles often involves hazardous solvents and generates significant waste. There is an urgent need for greener methods to obtain indole derivatives, both to reduce the environmental footprint and to manage pharmaceutical waste streams. Expired or unused **pharmaceutical drugs containing indole cores** represent not only a pollution concern but also an untapped resource that could be "upcycled" into new therapeutics. By recovering indole-based actives from such sources using eco-friendly techniques, this research addresses a dual need: environmental sustainability and efficient drug development.

Concurrently, **bioinorganic chemistry** offers a strategy to enhance or modulate the bioactivity of organic drug molecules by forming metal complexes. Transition metal ions (especially first-row d-block metals) can coordinate with organic ligands (such as indole-

containing drugs) to create novel compounds with potentially superior pharmacological profiles. Prior studies have shown that coordination of drugs to metals can improve properties like antimicrobial potency, antioxidant capacity, and even reduce side effects. For example, copper(II) complexes of indomethacin were found to mimic superoxide dismutase (antioxidant enzyme) activity while causing less gastrointestinal damage than indomethacin itself. Such findings highlight the promise of **indole–metal complexes** as multi-functional agents – combining the organic drug's activity with the metal's catalytic or biochemical roles. There is a compelling need to explore this synergy in a systematic way. In particular, indole-bearing drugs like indomethacin (anti-inflammatory) or tryptophan (nutritional supplement and precursor of neurotransmitters) can act as bidentate or multidentate ligands, coordinating metals through functionalities (e.g. carboxylate, amino, carbonyl groups) attached to the indole ring. **Green chemistry** principles should be interwoven throughout this work: from the benign extraction of the indole compounds to the solvent-conscious synthesis of their metal complexes. This ensures not only an environmentally responsible approach but also alignment with modern pharmaceutical development trends that demand sustainability. In summary, the present research is driven by the convergence of medicinal urgency and environmental responsibility – it seeks to **harness pharmaceutical indole scaffolds** and elevate their efficacy via metal coordination, all while employing green methodologies. This innovative approach could yield new bioactive complexes (addressing issues like antibiotic resistance and oxidative stress-related diseases) and set a precedent for repurposing pharmaceutical waste into value-added products, exemplifying the principles of circular chemistry.

b. Details of the Project with Chapterization

To address the above needs, the Ph.D. dissertation is structured into clear chapters, each covering a facet of the work from background to experimental results. The major chapters and their expected contents are outlined below:

- **Chapter 1: Introduction:**

  Indole is one of the most significant heterocyclic scaffolds in modern medicinal chemistry, widely distributed in pharmaceutical drugs, natural products, and biological systems. Its unique bicyclic structure imparts exceptional stability, bioavailability, and binding

versatility, enabling it to participate in diverse biochemical and pharmacological processes. Several clinically relevant drugs—such as indomethacin, tryptophan derivatives, and other indole-based anti-inflammatory and antimicrobial agents—demonstrate the importance of this core in drug design. Because of its privileged pharmacophoric properties, indole remains a central motif for developing therapeutics with enhanced activity, selectivity, and biological compatibility.

Parallel to the structural importance of indole derivatives, the principles of **green chemistry** have reshaped modern extraction and purification techniques. With increasing emphasis on sustainability, eco-friendly extraction approaches such as **microwave-assisted extraction (MAE)**, **ultrasound-assisted extraction (UAE)**, and **supercritical CO_2 extraction (SFE)** have gained prominence for isolating bioactive molecules from complex pharmaceutical matrices. Integrating green chemistry with medicinally relevant scaffolds like indole not only reduces environmental impact but also enhances extraction efficiency and product purity. Against this backdrop, the need arises to explore the extraction of indole derivatives through green methodologies, followed by their transformation into metal complexes for enhanced therapeutic potential.

- **Chapter 2: Literature Review**

  Indole chemistry has been widely explored owing to the structural and biological relevance of this heterocycle. Numerous studies highlight its presence in pharmacologically active compounds such as indomethacin, tryptophan-derived metabolites, and various natural products exhibiting anti-inflammatory, antimicrobial, and antioxidant effects. Literature consistently identifies indole as a **versatile pharmacophore**, capable of forming strong interactions with biological receptors due to its electron-rich aromatic system and conformational adaptability.

  Green extraction methodologies have also been well-documented in recent research. Techniques such as MAE, UAE, and SFE have been shown to significantly improve extraction yields, reduce solvent consumption, and minimize thermal degradation of sensitive bioactive compounds. The adoption of these environmentally responsible methods has accelerated the development of green protocols for isolating active pharmaceutical ingredients, including indole-containing drugs.

Another major area of research concerns the synthesis and biological evaluation of transition metal complexes of pharmacologically active ligands. Coordination of organic molecules—including indole derivatives—to metals such as Cu(II), Ni(II), Co(II), and Zn(II) often enhances antimicrobial, antioxidant, and cytotoxic properties. Reported studies demonstrate that metal–ligand coordination can modify redox characteristics, improve membrane permeability, and alter binding interactions with biomolecules. Specific attention has been given to complexes derived from indole frameworks, including metal–indomethacin and metal–tryptophan complexes, which exhibit improved biological profiles compared to their free ligands.

Despite the extensive literature on indole derivatives, green extraction techniques, and transition metal complexes, no integrated study has yet combined the green extraction of indole-based pharmaceutical drugs with the subsequent synthesis, characterization, and biological evaluation of their 3d-transition metal complexes. This critical gap underlines the novelty of the present research and positions it to contribute uniquely to the fields of medicinal inorganic chemistry, green extraction science, and drug–metal complex design.

- **Chapter 3: Green Extraction of Indole Derivatives from Pharmaceuticals** – This chapter details the development of environmentally friendly extraction protocols to isolate indole-based compounds from selected sources. The target compounds include **indomethacin** (isolated from tablet formulations or bulk drug powder) and **L-tryptophan** (isolated from supplement mixtures or protein hydrolysates). Three green extraction techniques will be optimized and compared: (1) **Microwave- Assisted Extraction (MAE)**, using microwave heating to rapidly liberate the active indole compound into a benign solvent (e.g. aqueous ethanol). Microwaves can dramatically reduce extraction time and solvent usage by heating the matrix internally, even for heat-sensitive compounds. (2) **Ultrasound-Assisted Extraction (UAE)**, employing ultrasonic cavitation in a solvent to disrupt matrices and enhance mass transfer. Ultrasound is considered a non-thermal process that avoids degrading thermolabile actives and accelerates extraction efficiency. (3) **Supercritical CO_2 Extraction (SFE)**, utilizing CO_2 at supercritical conditions (around 31 °C and 74 bar, possibly with co-solvents for polarity tuning) to dissolve and extract the indole derivative. Supercritical CO_2 is an attractive green solvent because it is non-toxic,

leaves **no solvent residue** in the product, and allows mild, tunable extraction conditions. In this chapter, the methodology for each technique will be described in detail: for instance, indomethacin tablets may be ground and subjected to MAE in a microwave reactor (e.g. 10–15 min irradiation at controlled temperature) versus UAE in an ultrasonic bath for various time intervals, versus SFE in a high-pressure apparatus. We will report the **optimization of key parameters** (microwave power and time; ultrasound amplitude and time; CO_2 pressure, temperature, co-solvent %) to maximize yield and purity. Yields of extracted indole (mg of indomethacin or tryptophan recovered per gram of source) will be compared among methods. We expect to demonstrate that the green techniques can achieve near-quantitative recovery of the active ingredient with far less organic solvent and energy than conventional solvent reflux extraction. This chapter will also discuss any practical challenges (e.g. need for a polar co-solvent in $scCO_2$ to extract indomethacin's carboxylate form) and how they were overcome. The outcome is a validated extraction approach that aligns with sustainability while providing sufficient material for the next steps.

A) Purification, Isolation, and Synthesis of 3d-Transition Metal Complexes of Indole Derivatives:

Following green extraction, the crude indole-rich extracts may contain impurities originating from excipients, co-extracted amino acids, or solvent components. This unified subsection outlines the complete downstream workflow — purification of isolated indole derivatives and their subsequent use in synthesizing 3d-transition metal complexes.

Purification and Isolation of Indole Derivatives:

Large-scale preparative HPLC is avoided due to high solvent consumption. Instead, flash column chromatography on silica gel is employed as a greener, scalable, and solvent-efficient method. Indomethacin extracts, for instance, may be purified using gradients of ethyl acetate/ethanol or greener solvent mixtures like acetone with alcohols, avoiding chlorinated or highly toxic solvents.

Purity will be assessed through analytical HPLC or TLC, and overall mass recovery will be documented. When applicable, recrystallization (e.g., indomethacin crystallized from

ethanol) will serve as an additional eco-friendly purification step. These purification steps produce gram-scale quantities of high-purity indomethacin and L-tryptophan, ready for coordination chemistry applications.

Synthesis of 3d-Transition Metal Complexes:

The purified indole derivatives are then used as ligands to form complexes with Cu(II), Ni(II), Co(II), and Zn(II)—metals of biological and pharmacological relevance.

The general synthetic method involves reacting the ligand (deprotonated when necessary) with a metal salt:

Indomethacin complexes:

Indomethacin is deprotonated using a mild base and reacted with metal salts such as Cu(II) acetate, forming indomethacinate complexes in aqueous or alcoholic media.

Tryptophan complexes:

$ZnCl_2$, $Zn(acetate)_2$, or corresponding Ni/Co salts are mixed with L-tryptophan at slightly basic pH, enabling coordination through amino and carboxylate functional groups.

Reaction conditions — solvent choice (favoring water/ethanol), temperature (room temperature to mild heating or ~60–70 °C reflux), and metal-to-ligand ratios (1:1 or 1:2) — are optimized. The resulting complexes often form colored precipitates (blue-green for Cu^{2+}; pink for Co^{2+}), which are filtered, washed with green solvents (water or ethanol), and dried. All yields will be recorded.

Exploratory green-chemistry methodologies such as microwave-assisted complex synthesis or natural deep eutectic solvents (NADES) will be noted if used.

Initial observations, including color changes, solubility variations, or shifts in absorption or pH, provide preliminary confirmation of complex formation.

Chapter 4: Characterization of the Metal Complexes – To confirm the structures and composition of the synthesized complexes, comprehensive **analytical characterization** will be carried out, detailed in this chapter. A combination of spectroscopic, analytical, and

diffraction techniques will be employed:

- *Fourier-Transform Infrared Spectroscopy (FTIR):* IR spectra of the free ligands (indole derivatives) versus their metal complexes will be compared. Key changes, such as shifts in the **C=O stretching frequency** of indomethacin's carboxylate or the N–H vibrations of tryptophan's amine, indicate
  coordination. For example, a carboxylate's asymmetric and symmetric stretch bands may shift and change in separation, reflecting monodentate vs bidentate binding to the metal. FTIR will thus confirm the presence of metal–ligand bonds (e.g. appearance of M–O or M–N stretches in the far-IR region).
- *Ultraviolet-Visible Spectroscopy (UV-Vis):* Electronic spectra of the complexes (in solution) will be recorded to observe d–d transitions and charge-transfer bands characteristic of each metal center. The d–d bands can provide insight into the coordination geometry (e.g. octahedral Cu(II) might show a broad band around 600–700 nm). Any ligand-to-metal charge transfer (LMCT) bands (for example, indole π to metal) would appear in the UV-visible region, confirming complex formation. UV-Vis spectroscopy will also be useful in quantifying complex stability (via Job's method or UV titration, though this might be beyond scope).
- *Nuclear Magnetic Resonance (NMR) Spectroscopy:* For diamagnetic complexes (notably Zn(II) complexes, and possibly Ni(II) if a square-planar d^8 complex is diamagnetic), ^1H and ^13C NMR will be acquired. Comparing the NMR of the free ligand to that of the complex can show coordination-induced shifts. For instance, the indole N–H proton or aromatic protons might shift upon metal binding; in tryptophan, the α-CH proton could shift if the amino group binds metal. Paramagnetic complexes (Cu(II), high-spin Co(II), Ni(II) octahedral) typically broaden NMR signals; thus for those, we may rely on NMR of the ligand in presence of metal (at low ratio) or simply confirm by other means. If needed, magnetic susceptibility measurements will be noted to confirm spin state (important for Ni and Co complexes).
- *Mass Spectrometry (MS):* To confirm molecular weights and stoichiometry, electrospray ionization (ESI-MS) or MALDI-TOF MS will be used. We will look for peaks corresponding to the metal–ligand compositions (e.g. for a Cu– Indomethacin 1:2 complex,

an m/z corresponding to [Cu(ligand)_2 – H]^+ or adduct ions). Isotopic patterns (especially for Cu, Zn which have distinct isotopes) can support the identification. MS can thus verify if complexes are mononuclear or perhaps dinuclear (e.g. some Cu(indomethacin)_2 complexes tend to form dimeric "paddle-wheel" structures, which might show a peak at a dimer mass).

- *Elemental Analysis (CHN analysis):* The percentage of carbon, hydrogen, nitrogen (and by difference, an estimate of metal content when combined with separate metal analysis) will be measured to confirm the empirical formula of each complex. Consistency of the found values with the calculated formula (within acceptable error) will indicate purity and the presence of any solvent of crystallization. For example, a complex formulated as Cu(Indomethacin)_2(H_2O)_1 might be confirmed by CHN % close totheoretical values and a noted loss of weight in TGA (see below) corresponding to one water molecule.
- *X-ray Diffraction:* **Single-crystal XRD** will be the definitive method for structural characterization if suitable crystals can be grown. We will attempt to crystallize at least one representative complex for each ligand (e.g., slow evaporation or diffusion methods to grow crystals of a copper–indomethacin complex). A solved crystal structure would reveal the coordination geometry (e.g. Cu–indomethacin forming a dinuclear carboxylate-bridged paddlewheel vs. Ni–indomethacin forming mononuclear octahedra). Even if single crystals are not obtainable for all complexes, **powder XRD** on the bulk solids will be done to check crystallinity and phase purity. Powder patterns can be compared to any reported structures or to patterns simulated from single- crystal data (if available).
- *Thermal Analysis (TGA/DSC):* Thermogravimetric analysis will assess the thermal stability of the complexes and help identify if any lattice solvents are present. For instance, a weight loss around 100 °C might indicate loss of coordinated or crystallization water (as observed in some indomethacin complexes which often include water in the coordination sphere). TGA coupled with differential scanning calorimetry (DSC) could also detect any decomposition or phase transition temperatures, which relate to stability. A stable complex might show no major weight loss until a high temperature (200+ °C).

In this chapter, the results from all these techniques will be synthesized to propose structures for each complex. For example, we expect the indomethacin complexes to exhibit

bidentate or monodentate carboxylate coordination modes; IR will differentiate these, and XRD (if obtained) will confirm it. The characterization ensures that the subsequent biological evaluation is performed on well-defined compounds. Any interesting findings (such as polymorphism, or differences between, say, Ni and Cu complexes in structure) will be discussed here.

- **Chapter 5: Biological Evaluation of the Ligands and Complexes** – The final experimental chapter examines the **bioactivity** of both the free indole derivatives and their metal complexes, with a dual focus on **antimicrobial** and **antioxidant** effects. The aim is to test the hypothesis that metal coordination enhances biological activity. This chapter is divided into two main sections:

- *Antimicrobial Assays:* We will evaluate the antibacterial and antifungal activity of the compounds in vitro. A panel of representative microorganisms will be used, likely including **Gram-positive bacteria** (*Staphylococcus aureus*, *Bacillus subtilis* or *B. cereus*), **Gram-negative bacteria** (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), and a **fungal strain** (*Candida albicans*). Initial screening will employ the **agar disc diffusion** method: discs infused with each test compound (free indole ligand vs. its metal complexes, at a fixed concentration) are placed on inoculated agar to observe zones of inhibition. Enhanced inhibition zones for the metal complex compared to the free drug would indicate improved potency. For example, if Cu–indomethacin shows a larger zone against *B. cereus* than indomethacin itself (as reported in similar studies), it suggests added antibacterial action due to the metal. Quantitative assays will follow, such as determining the **minimum inhibitory concentration (MIC)** using broth microdilution. The MIC (lowest concentration that inhibits visible growth) will be measured for each compound-organism combination. These results will be compared to a standard antibiotic (like ciprofloxacin or ampicillin as positive control) and to the free ligand. Any synergy or enhanced spectrum of activity upon metal coordination will be noted. It's expected, based on literature, that complexes of Cu(II) in particular may show significant antimicrobial effects since copper itself has known biocidal properties. If, for instance, Zn(II)–tryptophan or Ni(II)–tryptophan complexes exhibit activity where tryptophan (an amino acid, not an antibiotic) alone has none, that would be a remarkable finding turning an inert molecule into an antimicrobial agent via complexation.

- *Antioxidant Assays:* The ability of the compounds to **quench free radicals** orotherwise act as antioxidants will be assessed through chemical assays. A common method employed will be the **DPPH radical scavenging assay**. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that has a deep violet color; antioxidants can reduce it to a yellow diphenylpicrylhydrazine, with a corresponding decrease in absorbance at 517 nm. In our tests, solutions of each ligand and complex (at various concentrations) will be incubated with DPPH, and the absorbance decrease will be measured over time (e.g., after 30 minutes in the dark). From this, the percent radical
  scavenging can be calculated and an IC_{50} (concentration for 50% inhibition of DPPH) determined. **ABTS cation radical decolorization** is another assay that may be used for comparison. We will include known antioxidants (like ascorbic acid or **caffeic acid**) as positive controls. The expectation is that certain metal complexes – especially those of redox-active metals like Cu(II) or Co(II) – will show greater radical scavenging ability than the free indole compounds. This is because the metal center can participate in redox cycling to help quench radicals, or stabilize the deprotonated antioxidant form of theligand. Literature already indicates, for example, that Cu(II) and Co(II) indomethacin complexes scavenged DPPH more effectively than indomethacin itself (though not as strongly as dedicated antioxidants like caffeic acid). We will verify such trends for our synthesized complexes. If a complex demonstrates significantly improved antioxidant capacity (lower IC_{50}) relative to the parent drug, it suggests potential for therapeutic use in oxidative stress-related conditions.

All biological test results will be statistically analyzed (e.g., calculating mean ± standard deviation of triplicate measurements, and using t-tests to compare complex vs. ligand performance). This chapter will discuss the **structure-activity relationships** observed. For instance, we might find that among the metals tested, **copper complexes** show the highest antimicrobial effect (owing to copper's known antimicrobial mechanisms), whereas **zinc complexes** (with filled d-shell) might rely on the ligand's activity and show moderate improvements. Similarly, complexation might enhance indomethacin's antioxidant effect by a small margin, whereas tryptophan – normally not a potent antioxidant – could become more active when bound to, say, Cu(II) that can undergo redox cycling. Any lack of enhancement or reduced activity will also be noted (for example, if a complex is too

insoluble and thus bio-unavailable, it may show diminished apparent activity, underscoring the importance of solubility). Ultimately, Chapter 6 will identify which metal–indole complex has the most promising biological profile.

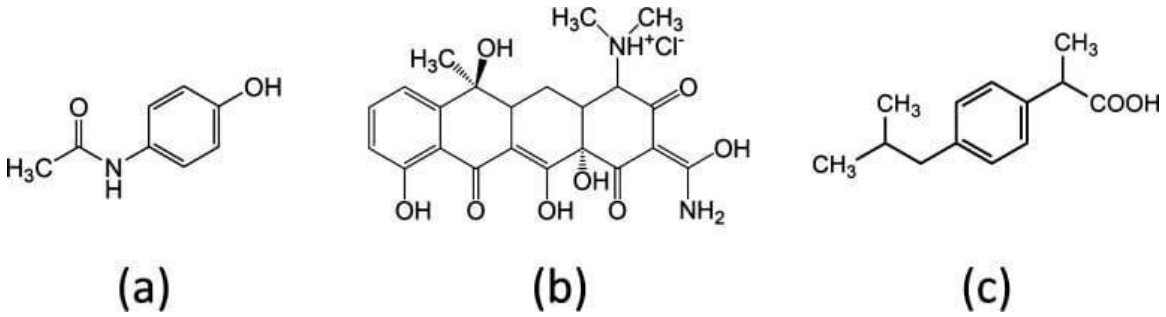
- **Chapter 6: Conclusions and Future Perspectives** – The final chapter will summarize the key findings of the research, tying back to the original hypotheses. It will highlight how the study successfully demonstrated a greener extraction of pharmaceutical indoles and the creation of novel metal complexes with tunable bioactivities. We will discuss the broader implications: for example, the feasibility of scaling green extraction for pharmaceutical waste recycling, or the potential of a particular Cu–indole complex as a lead for new antimicrobial agents. Limitations of the study (such as any challenges in crystallization or if some complexes had lower stability) will be candidly addressed. Finally, future directions will be suggested, such as exploring other metals (Fe, Mn) or testing the most active complexes in advanced biological models (cell culture or enzyme assays to understand mechanism), and extending the green chemistry approach to other drug scaffolds. This ensures the dissertation not only reports on the completed work but also paves the way for subsequent research building on these results.

c. Hypothesis

This research is guided by several interrelated hypotheses:

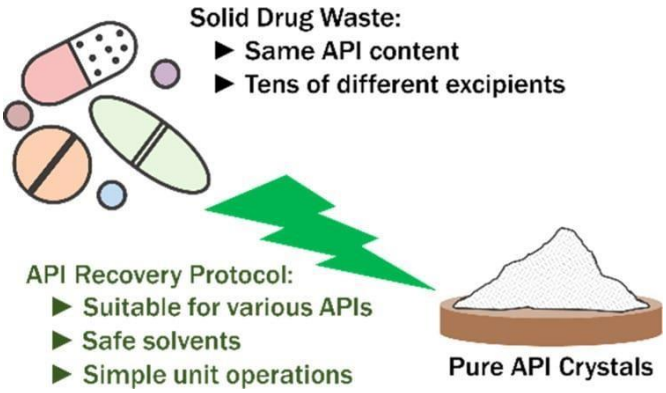
- **Greener Extractions are Feasible and Effective:** It is hypothesized that **green chemistry techniques** (microwave, ultrasound, supercritical CO_2) can **efficiently extract indole derivatives** (such as indomethacin and tryptophan) from pharmaceutical preparations or natural matrices with yields comparable to conventional solvent-intensive methods. In other words, environmentally benign solvents (e.g. aqueous ethanol or CO_2) and non-traditional energy inputs will recover the target compounds in high purity while **minimizing hazardous waste**. Success will support the idea that sustainable practices can replace classical organic solvent extraction for pharmaceutical compounds. This hypothesis stems from the expectation that microwave heating and ultrasonic cavitation can greatly accelerate solubilization of active ingredients, and that CO_2 will selectively solvate the indole compounds without leaving toxic residues.

Indole Derivatives:



- **Indole-Containing Drugs Serve as Effective Ligands:** We hypothesize that the **indole derivatives isolated (indomethacin, tryptophan, etc.) will act as bidentate or multidentate ligands**, coordinating strongly with 3d transition metal ions to form stable complexes. The indole scaffold, although not directly binding the metal, carries functional groups (like –COOH, –NH_2) that can chelate metals in a predictable manner. For example, indomethacin's carboxylate is expected to bind metal centers (possibly in bridging modes for some metals), and tryptophan can chelate via its amino and carboxylate groups. We anticipate isolating well-defined complexes with metal-to-ligand ratios such as 1:2 (common for many amino acid and drug complexes). This hypothesis will be supported if spectroscopic signatures (IR shifts, new UV-Vis bands) and analytical data confirm the formation of these **coordination compounds**.

Reported Procedure:



- **Metal Complexation Enhances Bioactivity:** The central hypothesis is that **coordination of indole-based drugs to transition metals will augment their biological activities**. We expect the metal complexes to exhibit **enhanced antimicrobial and antioxidant effects** compared to the free ligands. This is based on the rationale that the metal can introduce new mechanisms (e.g. generating reactive oxygen species that kill bacteria, or redox cycling for radical scavenging) and can improve pharmacokinetics (e.g. increased cellular uptake of a charged complex). For instance, a Cu(II)–indomethacin complex may combine copper's antibacterial properties with indomethacin's, yielding a broader spectrum or higher potency against microbes. Similarly, chelation might stabilize certain antioxidant forms of indole derivatives, leading to greater radical quenching ability. If our assays show significantly larger zones of inhibition or lower IC_{50} values for complexes versus the uncoordinated drugs, it will validate this hypothesis. We also posit that **different metals will impart different degrees of enhancement** (e.g., Cu complexes might outperform Zn complexes in antimicrobial activity due to Cu's biocidal nature, whereas Zn or Ni might still confer benefits but to a lesser extent).

- **Improved Safety and Stability:** An auxiliary hypothesis is that metal coordination could **reduce adverse effects or improve stability** of the drug. For example, indomethacin's gastrointestinal toxicity might be mitigated by complexation with Zn or Cu by modulating its release or targeting. While full pharmacological testing is beyond this project, any observations like a complex being less corrosive in a solvent or having a higher decomposition temperature (TGA) would support this idea.

- **Waste-to-Value Concept in Pharmaceuticals:** Underpinning the above, we hypothesize that our approach will demonstrate a viable **"waste-to-value" pathway**: instead of discarding indole-containing pharmaceuticals, they can be **recovered and transformed into new agents**. A successful outcome would be proof that using greener methods to extract a drug (like indomethacin) from expired pills and converting it into a metal complex with superior bioactivity is not only possible but advantageous. This validates a model of circular economy in the pharmaceutical sector – turning waste into value-added products, which is a novel element of this study.

Each of these hypotheses will be evaluated through the experimental work. Together, they

predict that the project will achieve a greener extraction process, obtain stable metal complexes, and find improved bioactivity, thereby confirming that sustainable practices and bioinorganic innovation can go hand-in-hand in drugdevelopment.

d. Methodology

Selection of Indole-Containing Drugs: The project will begin by choosing representative pharmaceutical compounds that contain an indole moiety and have known biological relevance. Two primary candidates are **Indomethacin** and **L- Tryptophan**. *Indomethacin* is a synthetic indole-acetic acid derivative (used as a nonsteroidal anti-inflammatory drug) – it provides a model of an indole-based active pharmaceutical ingredient (API) that we can attempt to recover from formulated products (e.g., capsules or tablets). *L-Tryptophan* is an essential amino acid with an indole ring, obtainable as dietary supplements or from protein sources; it represents a naturally occurring indole derivative with nutritional/pharmaceutical uses. These two were chosen to cover both a **drug molecule** (indomethacin) and a **bio-molecule** (tryptophan) as ligands. Both also possess functional groups suitable for metal coordination (indomethacin has –COOH and a carbonyl, tryptophan has $–NH_2$ and –COOH) and are expected to form stable complexes. Prior to extraction, any solid dosage forms (for indomethacin) will be ground to increase surface area, and if tryptophan is obtained from a mixture (like protein hydrolysate), the matrix (e.g. plant material or supplement powder) will be prepared accordingly.

Green Extraction Procedures: We will perform parallel extractions using three **green techniques** to isolate the indole compounds from their sources:

1. **Microwave-Assisted Extraction (MAE):** A laboratory microwave reactor (with controlled temperature and pressure) will be used. For indomethacin, for example, a weighed amount of ground tablets (or bulk drug powder mixed with excipients to simulate a tablet matrix) will be suspended in a solvent such as 70% ethanol (an environmentally benign solvent mixture). The suspension will be microwaved at a set power (e.g. 300 W) for a short duration (5–15 minutes) at a target temperature (50–60 °C). The microwave's rapid volumetric heating is expected to dissolve indomethacin efficiently out of the solid matrix. For tryptophan, which is water- soluble, an aqueous or ethanolic solvent can be

used similarly. The conditions (time, temperature, solvent) will be optimized by testing aliquots – monitoring how much indole compound is extracted (via UV or HPLC analysis of the extract) as conditions vary. We anticipate that MAE will significantly reduce extraction time and required solvent volume, based on its demonstrated ability to accelerate extraction of bioactives. After microwave treatment, the hot mixture will be cooled and filtered to separate the solid residue. The filtrate (extract) containing the indole derivative will be collected for further purification.

2. **Ultrasound-Assisted Extraction (UAE):** Here, an ultrasonic bath or probe sonicator will be employed. The sample (e.g., indomethacin tablet powder or plant material containing tryptophan) will be immersed in a solvent (likely similar
   solvents: aqueous ethanol or pure water for tryptophan if it's very polar). Ultrasonic waves (20–40 kHz) will be applied, creating cavitation bubbles that disrupt solid matrices and cell walls (if natural source), freeing the target compounds. We will sonicate for a set period (e.g., 10–30 minutes) at ambient or slightly heated conditions (ultrasonic energy can increase temperature to ~30–40 °C, which we can modulate by cooling if necessary). We'll optimize sonication time and amplitude; often, a moderate amplitude (e.g. 50–60%) is sufficient. Ultrasound is particularly useful to avoid high temperatures, thus preserving thermolabile indole compounds. After sonication, the mixture is handled like the MAE case – filtered to yield the extract solution. We expect UAE to be highly effective for tryptophan (as it's easily leached out) and also effective for indomethacin from pills (the agitation can help dissolve drug crystals into the solvent). This technique also uses relatively simple equipment and is easy to scale.

3. **Supercritical CO_2 Extraction (SFE):** For a solvent-free approach, supercritical CO_2 extraction will be attempted especially for indomethacin, which is moderately
   nonpolar (suitable for CO_2 solvating power when slightly polar modifiers are added). The sample (e.g. powdered indomethacin formulation) will be loaded into a stainless steel extraction vessel. Liquid CO_2 will be pumped in and heated to reach supercritical state (e.g., 40 °C and 120 bar to start). A small percentage of co-solvent (such as ethanol, ~5–10%) can be introduced if needed to enhance the extraction of polar indomethacin. The supercritical CO_2 will penetrate the solid and dissolve the indomethacin. After a set

extraction time (30–60 minutes of static/dynamic flow), the CO_2 carrying dissolved compounds will be expanded to gas (reducing pressure) into a collection vessel, where indomethacin will precipitate out (since CO_2 loses solvating power when back to gas). The collected material (or solution if a trap solvent is used) will contain the extracted indole derivative. We will optimize pressure, temperature, and co-solvent to maximize yield. Given CO_2's tunable solvent strength and clean nature, we anticipate obtaining high-purity indomethacin with no residual solvent. Tryptophan is quite polar and less likely to be extracted by neat CO_2; if attempted, a higher co-solvent fraction or an alternative approach (like ion-pairing with CO_2-philic counterion) might be needed. The viability of SFE for tryptophan will be assessed, but our main SFE focus is on indomethacin. We note that SFE is a **green technology** widely used in food/pharma because it leaves no toxic residues and operates at relatively low temperatures, protecting compound integrity.

For each method, the efficiency will be quantified: we will use analytical HPLC or UV spectrophotometry to determine how much indole derivative was extracted versus the initial amount (if known, e.g. a tablet containing 25 mg indomethacin – did we retrieve ~25 mg). The greener methods will be compared to a conventional extraction (e.g. Soxhlet or simple solvent reflux in dichloromethane) as a benchmark. We expect the green methods, particularly MAE and UAE, to yield equivalent or better extraction efficiency in a fraction of the time, fulfilling the first hypothesis of greener extraction success. Furthermore, these extracts (being in relatively benign solvents like ethanol/water) can be directly used in the next purification step without the need for solvent exchange.

Purification via Flash Chromatography: Once the crude extracts are obtained, they may contain impurities (for indomethacin: binders from tablets, for tryptophan: other amino acids if from a natural source, etc.). We will employ **flash column chromatography** to purify each compound. A medium-sized glass column or an automated flash system will be packed with silica gel (mesh ~40–63 μm). We will first test the crude extract on thin-layer chromatography (TLC) to identify a suitable solvent system that cleanly separates the indole compound from impurities. For indomethacin, a likely TLC solvent might be ethyl acetate:hexane or ethyl acetate:methanol; however, to keep it green, we'll consider **ethyl acetate:ethanol** or **acetone:water** mixtures, as these reduce toxicity. Based on TLC R_f

values, a gradient or step-gradient for flash chromatography will be designed. For instance, we could start with low polarity (to flush out very nonpolar excipients), then ramp to a more polar mix that elutes indomethacin. Flash chromatography allows large sample loading – we might load the entire extract (containing tens to hundreds of milligrams of product) in one go, unlike preparative HPLC which might require multiple injections.

Fractions from the column will be collected and analyzed (TLC or UV) to identify those containing the pure indole derivative. These fractions will be combined and solvent removed (via rotary evaporation) to yield purified indomethacin or tryptophan. Using flash chromatography in this way is anticipated to **reduce solvent usage** and time compared to preparative HPLC by at least 50%, aligning with greener purification goals (we will document the total solvent volume used). In case of tryptophan, since it's very polar and water-soluble, we might alternatively use an **ion-exchange resin** or gel filtration for purification, or crystallize it from water by adjusting pH (tryptophan has known isoelectric precipitation). But flash chromatography on a polar stationary phase (like C_{18} reversed-phase using water/ethanol gradient) is also an option for tryptophan. Any such alternate method will be considered if silica flash is ineffective. At the end of this step, we aim to have **analytically pure indole derivatives**. We will confirm purity by melting point (indomethacin has a sharp m.p. ~160 °C when pure) and single-spot TLC/HPLC. The yields of purified product from the initial material will be reported to assess the practicality of the green extraction plus flash workflow.

Synthesis of Metal Complexes: With pure indomethacin and tryptophan in hand, the next stage is to synthesize their complexes with Cu(II), Ni(II), Co(II), and Zn(II). We will follow general coordination chemistry protocols for complexation: - For each metal, an appropriate **metal salt** will be used. Likely choices: copper(II) acetate monohydrate (or $CuSO_4 \cdot 5H_2O$), nickel(II) chloride hexahydrate (or $Ni(OAc)_2 \cdot 4H_2O$), cobalt(II) chloride (or $Co(OAc)_2$), and zinc(II) acetate dihydrate. Acetate salts are often convenient as they are soluble in water/alcohol and the acetate can assist in deprotonating the ligand. -

Complexation with Indomethacin: Indomethacin (abbreviated HInd when protonated) is a monoprotic acid. To facilitate metal coordination, we will deprotonate it in situ by adding a slight stoichiometric excess of a base like sodium hydroxide or triethylamine in the presence of the metal salt. A typical preparation might be: dissolve $Cu(OAc)_2$ in ethanol-

water, separately dissolve indomethacin in minimal ethanol with a base, then mix the two solutions. The reaction might be done at room temperature or with gentle heating (50 °C) for 1–2 hours. The formation of a complex is often indicated by a color change (for Cu, a bright blue solution might turn green or dark blue as the indomethacinate binds). We will aim for a 1:2 metal:ligand ratio. The product may start precipitating during reaction (many metal-indomethacin complexes have limited solubility in water/ethanol). After allowing sufficient time for coordination, the mixture will be cooled (if heated) and the solid complex will be filtered off. It will be washed with a bit of cold solvent (perhaps a 1:1 water/ethanol mix to remove unreacted metal or ligand) and dried in vacuo. Each metal will be done similarly. If a metal (like Co(II)) has air-sensitivity (Co(II) can oxidize to Co(III) in presence of O_2), we will conduct the synthesis under inert atmosphere for that step or use excess ligand to keep Co(II) stable. - **Complexation with Tryptophan:** Tryptophan (HTrp) is an amphoteric zwitterion; in neutral solution it exists as H_3N^+-Trp-COO^-. For metal binding, a slightly basic environment (around pH 7.5–8.5) is ideal so that the amine is deprotonated ($–NH_2$) and carboxylate is deprotonated ($–COO^-$), enabling bidentate coordination. We will dissolve tryptophan in water (it's fairly soluble) and add, say, $Zn(OAc)_2$ solution slowly. We expect a complex like [Zn(Trp)_2(H_2O)_2] to form (as known in literature). The solution might be heated mildly to ensure completion. As Zn(II) complexes with amino acids can sometimes remain in solution, we might need to induce precipitation by adjusting solvent polarity (e.g., add ethanol to the aqueous solution) or by concentrating the solution.

Alternatively, we can perform the reaction in 50% ethanol where the complex might be less soluble and crystallize out. Each metal with tryptophan will be done similarly, keeping the pH controlled (using NaOH or buffer) to maximize chelation. If solids form, they are filtered; if not, we may fully evaporate the solvent and then triturate the residue with a non-solvent (like isopropanol or ether) to obtain the solid complex. - We will also explore **reflux or microwave synthesis** for these complexes if standard conditions give low yield. For example, microwave heating of a metal salt-ligand mixture can sometimes produce crystalline products quickly. Such experiments will be documented if attempted (e.g., microwave a mixture of $CoCl_2$ and indomethacin in ethanol for 10 minutes and observe complex formation). - **Product isolation and handling:** The isolated complexes

(particularly those of indomethacin) might incorporate solvent or water. We will note if the solid appears hygroscopic or if drying causes loss of weight (checked by TGA). We'll store them in dry conditions. Each isolated complex will be assigned a code (like Cu-Ind, Ni-Ind, Cu-Trp, etc.) and yields will be calculated. A successful synthesis is indicated by nearly quantitative conversion (e.g., recovery of ~ >80% of theoretical yield). If a particular metal does not form a precipitate (e.g., some Zn or Ni complexes might remain very soluble), we may resort to alternative methods like adding a precipitating anion (e.g., $NaPF_6$ to precipitate a complex cation as PF_6^- salt) or use lower temperatures to crystallize.

The methodology ensures that for each target complex we have an optimal route. We may find that some metals form multiple stoichiometries (as the conductometric study by Alayyafi et al. suggests Cu-Indomethacin can form 1:1 or 1:2 complexes in different conditions). In such cases, we will attempt to isolate the predominant form by controlling the metal:ligand ratio or by recrystallization. We will also apply **green chemistry principles** here: using water or ethanol as solvents, avoiding toxic solvents (no chlorinated solvents unless absolutely necessary for solubility, which is unlikely in these salt-based syntheses), and possibly using techniques like **ultrasonic agitation** to assist complexation (ultrasound can speed up dissolution of ligands and mixing of reactants, potentially yielding the complex faster – we will try sonication for some reactions to compare). By the end of this synthetic methodology, we expect to have a suite of at least 8 new complexes (Indomethacin + 4 metals, Tryptophan + 4 metals). These will then proceed to characterization.

Characterization Techniques: The methodology for characterization was broadly outlined in the chapter plan, but here we detail how each will be executed in practice: - *FTIR:* Samples (ligands and complexes) will be analyzed as KBr pellets or ATR (attenuated total reflectance). We will specifically look for diagnostic changes: for indomethacin, the free acid has a C=O around 1715 cm^{-1}; upon complexation (deprotonated carboxylate), this band might shift to ~1590 cm^{-1} (asymmetric) and ~1390 cm^{-1} (symmetric), with $\Delta\nu$ indicating monodentate vs bidentate coordination (monodentate typically has larger $\Delta\nu$). We'll compare those to literature values or to standard sodium indomethacinate spectrum if available. For tryptophan, N–H and O–H stretches (~3000 cm^{-1} region) may diminish when coordinated (especially if deprotonated). IR will be done

immediately after drying samples to avoid misinterpreting O–H from water unless water is part of structure (which TGA/XRD will clarify). - *UV-Vis:* Solutions of the complexes (e.g., in DMSO or a bit of water/methanol ifthey dissolve) will be scanned from 800 nm to 200 nm. If solubility is an issue, diffuse reflectance UV-Vis on solid samples can be an alternative (using an integrating sphere).

We'll record any visible bands (d–d transitions for Ni(II) might show green color (~400 nm band for Ni, Co(II) pink complexes might have weak d–d around 500 nm, Cu(II) often a broad band ~600–700 nm). For Cu-Indomethacin, if it forms dimeric species, there might be characteristic charge-transfer bands. These spectral features will be compared with known complexes (e.g., literature for Cu(Indomethacin)_2 which is green due to a strong dimer band around 700 nm). - *NMR:* ^1H NMR spectra of indomethacin (free) and Zn–indomethacin (for example) will be taken in deuterated DMSO (indomethacin is DMSO-soluble). We'll check if the indole N–H proton (around 10–11 ppm normally) disappears or shifts (it might still be present if not directly coordinated, but could shift if hydrogen-bonded to something in the complex). The aromatic protons (7–8 ppm) and the $–CH_3$ of the indole ring (around 2.5 ppm) could shift slightly. For tryptophan, free in D_2O, it shows an α- CH at ~3.5 ppm and aromatic multiplets ~7.2–7.6 ppm. In a Zn(Trp)_2 complex, these might shift or split if the environment is asymmetric. Paramagnetic complexes (Cu, Co high-spin, Ni high-spin) won't be analyzed by solution NMR due to broadening; instead, we might attempt solid-state ^13C or ^15N NMR if equipment allows, but that is optional. We will likely rely on other data for those. - *MS:* We'll use ESI-MS in both positive and negative mode (since some complexes might form anionic species like $[M(ligand)_2 – H]^-$ or cationic adducts $[M(ligand)_2 + Na]^+$). For example, for Cu(indomethacin)_2, an expected m/z in negative mode might be at the molecular weight minus a proton (because it's often isolatedas a neutral or anionic complex with a proton maybe balancing charge). If adducts form with Na or K, those will be considered. Similarly, Cu(Trp)_2 might appear as an M^+ (since tryptophan has a zwitterion nature, the whole complex might be neutral; but in ESI, one could get $[M + H]^+$ or $[M + Na]^+$). We will compare observed masses with calculated values to confirm stoichiometry. MS can also hint if one metal forms a dimeric complex (e.g. a peak at double the monomer minus some fragment might show up). - *Elemental analysis:* Samples (~2–3 mg) will be sent for CHN analysis. We'll interpret the

results to confirm formulas like $M(L)_2 \cdot xH_2O$. If, say, a copper complex analysis is slightly low in carbon/hydrogen, it might indicate presence of a water molecule (which adds H and O mass not counted in theoretical anhydrous formula). We'll iterate formula assignments until the analysis matches, complementing with the TGA findings. - *XRD:* We will attempt to grow single crystals by slow evaporation of a suitable solvent. For example, dissolve the Zn–tryptophan complex in hot water, let it cool slowly to crystallize, or use a diffusive layering (ether over a DMF solution) for indomethacin complexes. If crystals are obtained, single-crystal XRD will be done using a diffractometer (perhaps a low-temperature (100 K) data collection to get good precision). The structure solution will yield bond lengths M–O, M–N, etc., directly confirming coordination modes. In absence of single-crystal data, we will collect powder XRD of each complex and compare the patterns qualitatively – distinct sharp peaks indicate a crystalline phase, which could be matched to simulated patterns if we have partial structural info. Broad or amorphous patterns might occur for less crystalline solids (some metal-organics are amorphous). Either way, the crystallinity and any phase identity (especially if multiple forms exist) will be noted. - *TGA:* Using a thermogravimetric analyzer, we'll heat each complex (perhaps from room temperature to ~800 °C at 10 °C/min under air or nitrogen). We expect initial weight losses corresponding to solvent/water release (e.g., a 5% weight loss around 100 °C could signify one water per complex). Subsequent weight loss steps would correspond to decomposition of the organic ligand, leaving behind metal oxides at the end (the residual weight at high temperature should correlate with the metal content if it converts to, say, CuO or ZnO). TGA curves will thus support the proposed formula (especially the hydration number). Differential Scanning Calorimetry (if simultaneously recorded) might show endotherms for dehydration or exotherms for decomposition which we can report.

Through this meticulous characterization plan, we ensure the identity and structure of our complexes are well-established before biological tests. We will maintain a record of every characterization result in the dissertation, often including spectral graphs, XRD structures, etc., in this chapter.

Biological Assays Implementation:

- *Antimicrobial tests:* We will follow standard microbiology protocols. After preparing stock

solutions of each compound (likely in DMSO or sterile water if soluble), we'll do **disc diffusion** first for a qualitative assessment. Sterile filter paper discs (6 mm) will be impregnated with a fixed amount of compound (e.g., 50 µg per disc) and placed on Mueller-Hinton agar plates swabbed with a standardized bacterial inoculum (0.5 McFarland standard). After incubation (24 hours at 37 °C for bacteria, 48 hours at 30 °C for *Candida*), the inhibition zone diameters will be measured. We'll include discs of standard drugs (like ciprofloxacin for bacteria, nystatin for fungus as positive controls) and a blank DMSO disc as negative control. If complexes show any zones larger than the free ligand, that's a positive sign. We expect, for instance, Cu–indomethacin might show a clear zone against *S. aureus* whereas indomethacin might show none (since indomethacin is not a strong antibiotic itself). Following this, we will perform **MIC determination** by broth microdilution in 96-well plates. Serial two-fold dilutions of each compound will be prepared in nutrient broth (with DMSO kept below 2% v/v to avoid affecting microbes). Each well is inoculated with

~10^5 CFU/mL of the test organism and incubated overnight. The MIC is read as the lowest concentration with no visible growth (which can be confirmed with a resazurin dye indicator if needed). This will give a numeric value (e.g., MIC = 64 µg/mL for Cu-Indomethacin vs 256 µg/mL for indomethacin, hypothetically). Lower MIC indicates higher potency. We'll do this for at least a couple of representative bacteria (perhaps one Gram+ and one Gram−) and *C. albicans*. All tests will be done in triplicate for reliability. If any compound shows notable antimicrobial activity, we might additionally do a **time-kill curve** or attempt to observe its effect on biofilm formation (those are extended studies and may be future work, but mention if relevant). We will also observe if complexes are bacteriostatic or bactericidal (subculturing from MIC wells that showed no growth to see if bacteria are dead or just inhibited).

- *Antioxidant tests:* In the lab, we will prepare a fresh DPPH solution in methanol (with absorbance ~0.9 at 517 nm). Then in a set of test tubes (or a 96-well plate format), we'll mix DPPH solution with various concentrations of each test compound. For example, make solutions of ligand and complex at 10, 50, 100, 200 µM and add DPPH. After 30 minutes in dark (standard time for reaction), measure absorbance at 517 nm. We'll calculate % radical scavenged using the formula % scavenging = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$. Plotting

this against concentration allows IC_{50} determination. We'll do this for indomethacin vs its complexes, and tryptophan vs its complexes. Indomethacin may have slight antioxidant effect inherent (as some NSAIDs do scavenge radicals weakly); tryptophan might have minimal effect. We hypothesize complexes like $Cu(Indomethacin)_2$ will show greater % scavenging at the same concentration, so its IC_{50} will be lower. The presence of a metal like Co or Cu often introduces redox activity that can quench radicals (although sometimes it can also generate radicals via Fenton chemistry – but DPPH assay specifically measures net quenching). We will also use an ABTS assay as a confirmatory method: $ABTS^{\bullet+}$ (blue-green radical cation) is generated (with persulfate oxidation of ABTS) and compounds are tested for their ability to decolorize it to ABTS (colorless). The procedure is similar – measure absorbance at 734 nm. These antioxidant assays, along with controls (Trolox, a water-soluble vitamin E analog, as a positive control standard for antioxidant capacity), will allow us to compare the **antioxidant potency**. We'll likely express results in terms of IC_{50} (for DPPH) and maybe Trolox Equivalent Antioxidant Capacity (TEAC) for ABTS. If a complex has significantly better activity, we might inspect whether the metal's known chemistry explains it (e.g., Cu(II)/Cu(I) cycle helping to reduce the radical efficiently).

Throughout the methodology, **safety and green principles** will be observed: we will minimize use of toxic reagents (e.g., no heavy metals other than those of interest, and those chosen are relatively environmentally benign in small scale). Waste solutions containing transition metals will be collected and treated (precipitated as hydroxides for disposal). The overall experimental plan is designed to test our hypotheses stepwise: from obtaining the ligands in a green way, through synthesizing complexes, to evaluating enhancements in activity. A flowchart of this methodology might be presented in the thesis to illustrate how each part connects (from extraction to purification to synthesis to testing).

By executing this plan, the project will yield both tangible products (new metal complexes) and valuable data on the impacts of greener processes and metal binding on drug performance. Each methodological step is geared to produce results that either confirm or refute the hypotheses, thereby contributing to the final analysis.

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